Effects of Sublethal Concentrations of Cadmium on Adult *Palaemonetes pugio* Under Static and Flow-through Conditions

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Mortality studies of adult organisms over a given period of time have generally been the only standard bioassay techniques in pollution studies. Consequently, such techniques have played a major role in setting standards. It has also been shown that sublethal, thermal or chemical pollution can modify physiological and behavioral responses of organisms (DECOURSEY and VERNBERG 1972; HOSS *et al.* 1974; VERNBERG *et al.* 1973). These sublethal effects are often more difficult to assess than immediate lethality, but the long-term chronic exposure to sublethal solutions may markedly alter the normal functioning of organisms and thus destroy a population as effectively as a single lethal dose. Hence, it is imperative that techniques also be developed for detecting the effects of a sublethal concentration of a toxicant.

In measuring the impact of sublethal concentrations of a pollutant on organisms, it is essential to standardize the bioassay. Factors such as environmental regimes, stage in life history, sex, and body size may either singly or synergistically affect toxicity (DECOURSEY and VERNBERG 1972 ; VERNBERG and VERNBERG 1972; VERNBERG *et al.* 1973; BOOKHOUT *et al.* 1972; STRUHSAKER *et al.* 1974). Therefore, in measuring the impact of sublethal concentrations of a pollutant on organisms, all these factors must be considered. To establish a uniform bioassay procedure for sublethal concentrations of cadmium in a typical estuarine species, a series of studies was carried out with the grass shrimp *Palaemonetes pugio*, a common euryhaline estuarine species with a wide distributional range from Massachusetts to the Gulf Coast. Survival, molting time and metabolism were the measurement parameters for adult male grass shrimp maintained under different thermal-salinity regimes in either a static or a flow-through system, using $CdCl_2$ at different concentration levels as the toxicant.

MATERIALS AND METHODS

Shrimp were collected from the North Inlet Estuary near Georgetown, South Carolina during December, January, and February then transported immediately to the laboratory and housed in plastic boxes. Males were used in these experiments to eliminate possible sex difference in responses. Shrimp were acclimated to either cold (15^{o}C) or warm (25^{o}C) conditions for not less than 10 days, nor more than 3 weeks.

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For the static series, shrimp were housed individually in compartmentalized plastic boxes at either 15°C or 25°C, in 5, 10, 15 and 30 ‰ S on a 12L-12D light schedule. The water was changed every other day and the shrimp were fed newly-hatched *Artemia* nauplii at that time. The initial 50 ppb concentration of Cd was replenished with each water change.

Flow-through chambers were designed and built capable of accommodating twenty animals for either control or experimental situation (Fig. 1).

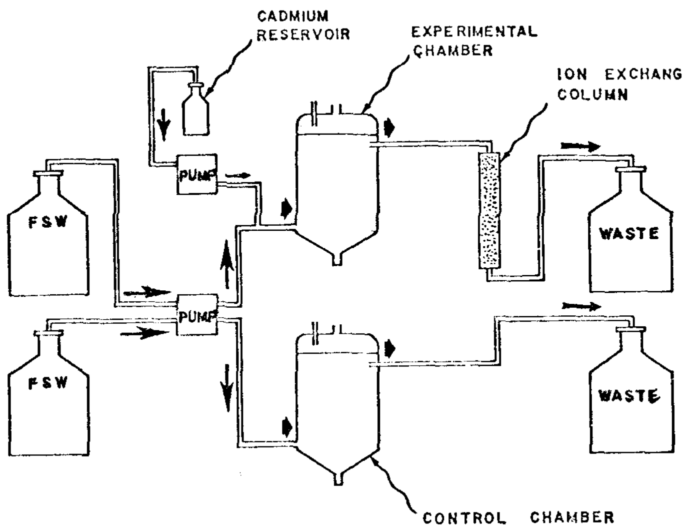

Figure 1. Flow-through System

The feeding regime paralleled that in the static system. Concentrated $CdCl_2$ (2.2×10^{-5} M) was dispensed from the cadmium reservoir by a Buchler polystatic pump and maintained the cadmium concentration in the flow-through vessels at approximately 50 ppb through the experiments. Temperatures were held at either 25°C or 15°C in salinities of 5, 15 and 30 o/oo S for 7 days.

Oxygen uptake rates of 10-18 shrimp (see Table 1 for group size) in static and flow-through systems were measured individually in a Gilson Respirometer after 7 days exposure to each experimental condition: 25° or 15°C acclimated at 5, 15 and 30 o/oo S. Subsequently the animals were rinsed with distilled water, then dried to a constant weight at 80°C. Results are expressed as µl O_2/hr/gm dry weight. Since a relatively wide size range of shrimp were used (0.007-0.07) respiration data were analyzed using linear regression techniques, and respiration rates were calculated from the linear regression curves for medium-sized (0.032 gm) shrimp.

TABLE 1

Oxygen consumption rates of control and cadmium-exposed *Palaemonetes pugio* from static and flow-through system, after warm or cold acclimation. Rates were determined after 7 days in test conditions. The data were calculated from the linear regression curves for medium-sized (0.032 gm) shrimp. Significant differences between static and flow-through linear regressions are noted: a) 10% level, b) 2.5% level.

CONTROL		warm-acclimated (25°C)		
Static			Flow-through	
Salinity °/oo	# Det.	uls O_2/hr/ gm dry wt.	# Det.	µls O_2/hr/ gm dry wt.
5	12	2282	9	2674
15	17	2446	18	2448
30	7	1279	20	1829
		cold-acclimated (15°C)		
5	16	1409	11	1078
15	9	818	16	1268
30	7	1504	10	1192
CADMIUM-EXPOSED		warm-acclimated (25°C)		
5	14	1850	9	3058
15	16	2269	22	2404
30[a]	8	1243	24	1916
		cold-acclimated (15°C)		
5[b]	17	644	5	1178
15[b]	9	1493	13	1408
30	16	1089	15	1147

Significant differences were calculated between linear regressions of control and experimental regimes as well as between static and flow-through regimes.

Survival of shrimp maintained in the static system at 25°C was monitored under salinities of 5, 10, 20 and 30 °/oo S using 20 freshly-caught shrimp for each salinity. Molting frequency for these shrimp was recorded, then analyzed with the Student's "t-test".

Cadmium concentration in shrimp tissues was analyzed using the dithizone method (STANDARD METHODS 1971). Three or four shrimp were used for each determination, and results expressed as ppm dry weight. Under static conditions, tissue concentration was measured after 3, 7, 14 and 21 days exposure at 25°C in 5, 10, 15, 20 and 30 °/oo S, and after 7 days exposure in 15°C at 5, 15 and 30 °/oo S. In the flow-through system, cadmium levels were determined after 7 days at 15°C or 25°C in 5, 15 and 30 °/oo S. Linear regression analysis was used to present the data graphically.

RESULTS AND DISCUSSION

Mortality for shrimp under control conditions in the static system was very low in all salinities. The increased mortality of the cadmium-exposed shrimp was related to the salinity regime; as salinities decreased, mortality increased. The level of Cd used in these experiments (50 ppb) is higher than that found in many marine waters. However, the level of cadmium reported from polluted estuaries have ranged as high as 78 ppm in Gulf Coast waters (HOLMES *et al.* 1974) and up to 9 ppb in the Bristol Channel in England (ABDULLAH 1974). *P. pugio* is able to tolerate relatively high levels of Cd (Fig. 2). Even at concentration levels of approximately 23 ppm, mortality rates were only 10%. Not all marine species are so resistant to Cd. Survival of *Uca pugilator* larvae, for example, was shortened upon exposure to a Cd concentration of 1 ppb (VERNBERG *et al.* 1974).

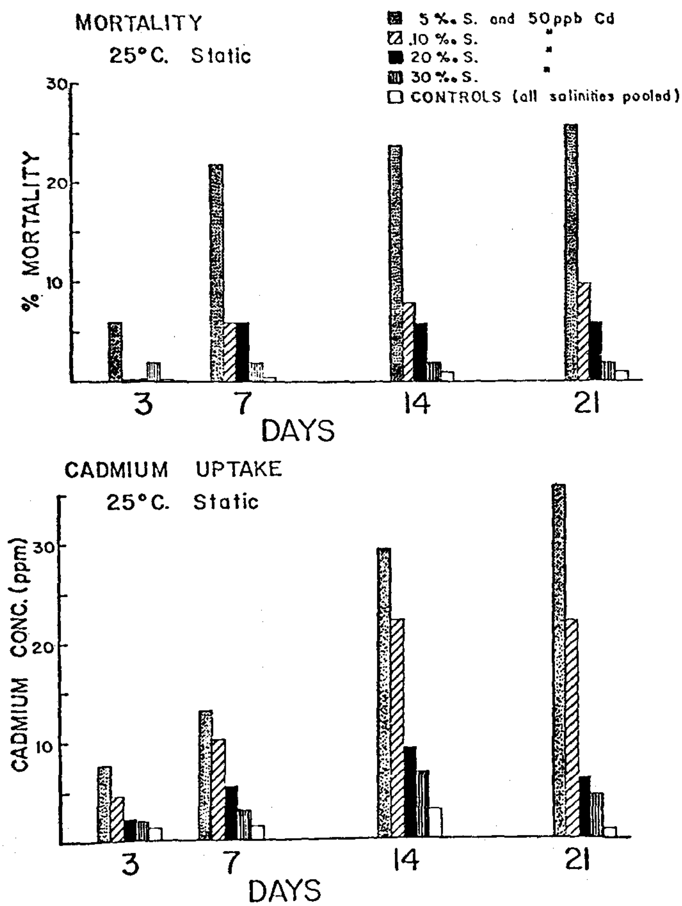

Figure 2. *Palaemonetes pugio*

Tissue accumulation of cadmium under both static and flow-through conditions was proportional to salinity at 25°C (Fig. 3). Highest cadmium concentrations for the static system occurred at 5 and 10 °/oo S; for example, after 21 days the experimental shrimp in 5 °/oo S contained approximately 35 ppm, while those at 10 °/oo contained only 20 ppm. Cadmium uptake rates in the static system differed from the flow-through system in some respects. Uptake levels were greater in shrimp from the flow-through system at 25°C after 7 days, although differences in the slopes indicating uptake levels are not statistically significant. At 15°C, less cadmium is concentrated than at 25°C in both static and flow-through systems, but shrimp in the flow-through system accumulated much more cadmium than those maintained under static conditions (Fig. 4), and the slopes indicating accumulation at this temperature were significantly different in the 2 systems. Uptake levels

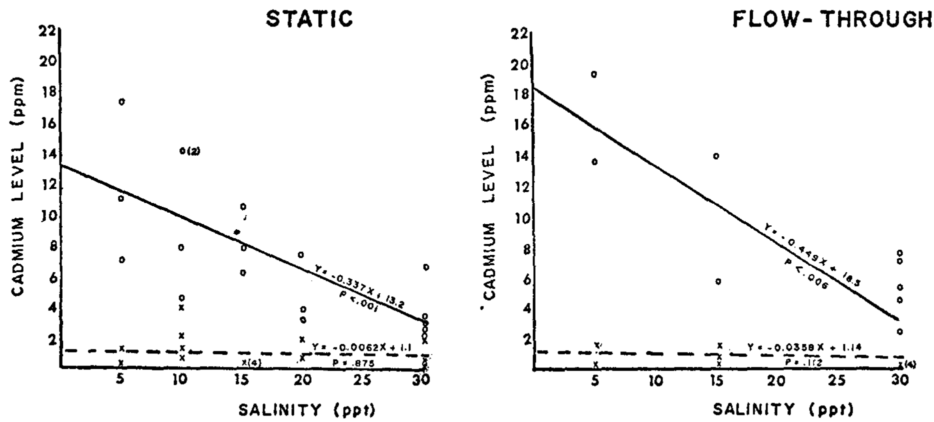

Figure 3. 7-day cadmium uptake levels vs salinity at 25°C.

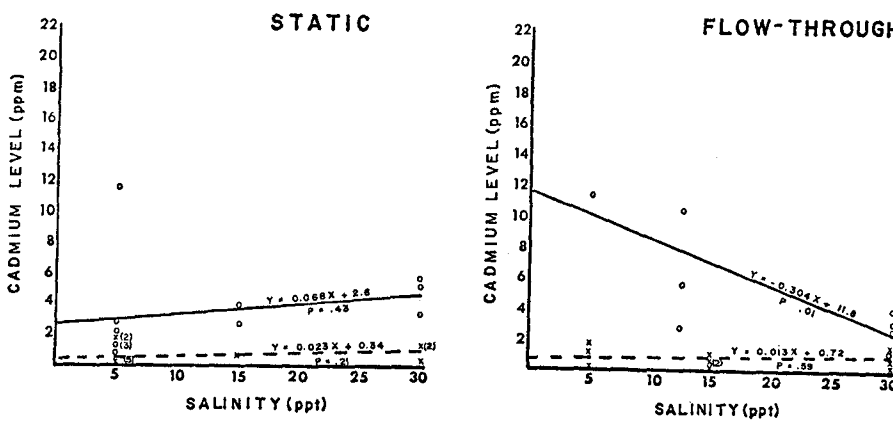

Figure 4. 7-day whole-body cadmium uptake levels vs salinity at 15°C.

of shrimp in the static system also did not show any salinity dependence while uptake of shrimp maintained in the flow-through system followed the same apttern observed at 25^{o}C and increased with exposure to low salinity. Similar results were reported by O'HARA (1973) for fiddler crabs; HUTCHINSON (1975) for blue crabs, and WESTERNHAGEN, ROSENTHAL, SPERLING (1974) for herring eggs.

At high body burden levels, as is found in animals kept at 5 ^{o}/oo S where the cadmium burden reached 40 ppm, cadmium inhibited molting. At more moderate levels, as was observed at 10 and 20 ^{o}/oo S with cadmium levels of 23 and 10 ppm respectively, molting was stimulated by the presence of cadmium (Fig. 5). It

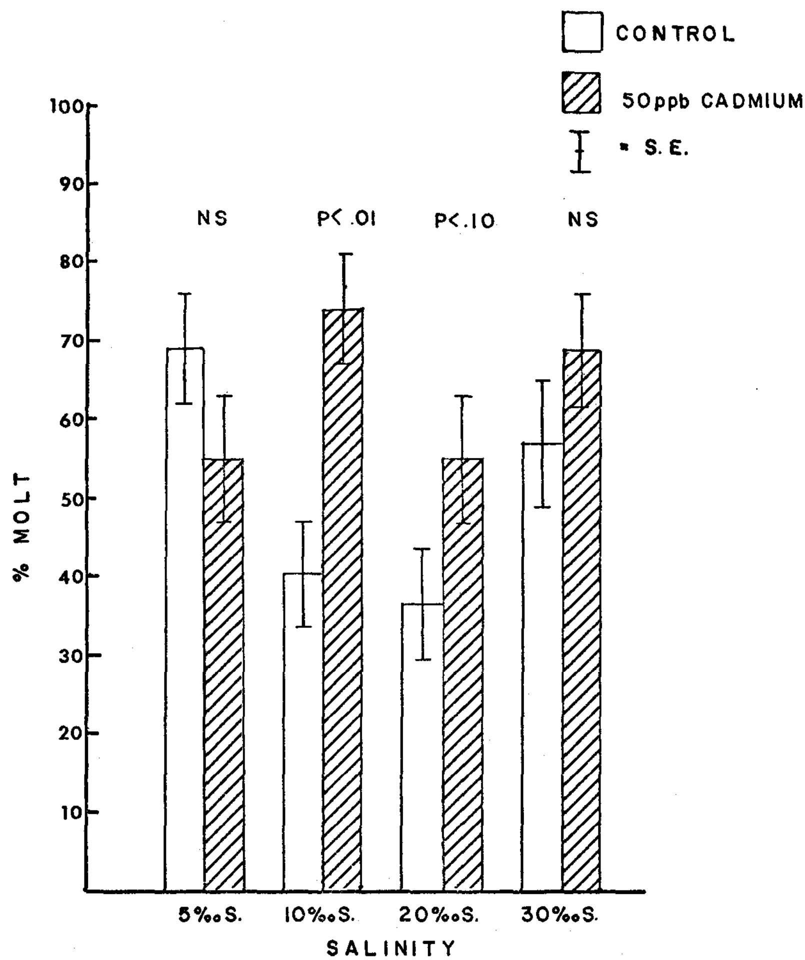

Figure 5. Molting frequency at 25^{o}C (static).

would appear that molting is affected only when fairly large amounts of cadmium have accumulated in the body, and up to a certain level it stimulates molting. Beyond that level, however, molting frequency is less than in control animals.

Respiration of cadmium-exposed shrimp tended to be higher in shrimp kept in the flow-through system than in the static condition (Table 1). Statistically significant differences were noted between the static and flow-through linear regression at 25°C, 30 °/oo S and at 5 and 15 °/oo S at 15°C, but there were no significant differences in linear regressions of static and flow-through systems under control conditions (Table 1).

A comparison of linear regressions of experimental and control shrimp maintained under static conditions showed significant differences under conditions of 25°C and 5 °/oo S; at 15°C significant differences were observed at both high and low salinity, 5 and 30 °/oo S. With both cold- and warm-acclimated shrimp, rates of those exposed to cadmium were lower than those of unexposed ones. On the other hand, no significant differences were noted under any temperature salinity regimes between control and experimental shrimp maintained in the flow-through system. The observed responses are all the more puzzling since more Cd was accumulated to a greater extent in shrimp from the flow-through system than under static conditions. Since the effect of Cd upon the shrimp is so different in the two systems, it is difficult to draw conclusions about metabolic-temperature responses in Cd-exposed animals, but these data suggest that the observed differences between control and experimental shrimp from the static system are due to factors other than Cd accumulation. In a study on the effects of Cd on the respiratory rate of another crustacean, *Eurypanopeus depressus*, COLLIER *et al.*(1973) came to a similar conclusion. The lack of clearly significant differences in respiration rates between control and experimental groups in both the flow-through and static systems probably represents at least in part the wide variation in activity that is exhibited by *P. pugio*. WELSH (1975) found a wide range of oxygen uptake rates when this species of shrimp was run at various temperatures. She attributed the variation to spontaneous changes in levels of activity. For example, the respiration rates at 18°C were so varied that they could not be statistically distinguished from those determined in temperatures ranging from 10°C to 30°C. Respiratory rates for another species of grass shrimp, *P. vulgarus*, also have been correlated with activity by MCFARLAND AND PICKENS (1965), who found that oxygen consumption rates determined for shrimp during sustained swimming were higher than those of shrimp subjected to enforced quiescence.

Data presented here suggest several conclusions. First, levels of cadmium must be relatively high to alter molting frequency or to threaten survival of the animal. There are little data available on concentrations of cadmium in *P. pugio* in nature. However, recently it has been reported that cadmium levels ranging from 10 to 20 ppm have been found in zooplankton collected off Baja, California (MARTIN and BROENKON 1975). Thus it is not inconceivable that high levels of cadmium could be present in natural populations of *P. pugio* and at these levels it is likely that the ability to survive environmental stress would be lessened in comparison to those shrimp in which cadmium levels were much lower.

Second, respiratory rates are not a predictable and reliable indicator of cadmium pollution in *P. pugio* because of the observed variability.

Third, there are differences both in metal uptake and in respiratory rates between animals maintained in a static system and those in a flow-through system. If, as seems likely, a flow-through system more nearly approximates field conditions than a static system does, data obtained from a flow-through system would be more applicable to field situations than data based on a static system.

Finally, *P. pugio* is too resistant and tolerant a species to be of value for short-term bioassay studies on cadmium.

REFERENCES

ABDULLAH, M. E. J. Mar. Biol. Assoc. U. K. *54*, 581-597 (1974).

BOOKHOUT, C. G., A. J. WILSON, Jr., T. W. DUKE, and J. I. LOWE. Water, Air and Soil Pollution *1*, 165-180 (1972).

COLLIER, R. S., J. E. MILLER, M. A. DAWSON and F. P. THURBERG. Bull. Envir. Contam. and Toxicol. *10*, 378-382 (1973).

DECOURSEY, P. J. and W. B. VERNBERG. Oikos *23*, 241-247 (1972).

HOLMES, C. W., E. A. SLADE, and C. J. MCLERRARE. Envir. Sci. and Tech. *8*, 255-259 (1974).

HOSS, D. E., L. C. CASTON and W. C. SCHAAF. Estuarine and Coast. Mar. Sci. *2*, 323-328 (1974).

HUTCHINSON, M. Ches. Sci. *15*, 237-242 (1975).

MARTIN, J. H. and W. W. BROENKON. Science *190*, 884-885 (1975).

MCFARLAND, W. N. and P. E. PICKENS. Can J. Zool. *43*, 571-585 (1965).

O'HARA, J. Fish. Bull. *71*, 149-153 (1973).

STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 13th edition. American Public Health Association, Washington, D. C. 874 pp.

STRUHSAKER, J. W., M. B. ELDRIGE, and T. ECHEVERRIA. *In* Pollution and Physiology of Marine Organisms. Academic Press, New York 1974.

VERNBERG, W. B., P. J. DECOURSEY, and W. J. PADGETT. Mar. Biol. *22*, 307-312 (1973).

VERNBERG, W. B., P. J. DECOURSEY, and J. O'HARA. *In* Pollution and Physiology of Marine Organisms. Academic Press, New York 1974.
VERNBERG, W. B. and F. J. VERNBERG. Fish. Bull. *70*, 415-420 (1972).
WASTERNHAGEN, H. VON, H. ROSENTHAL, K. R. SPERLING. Helg. wiss. Meere. *26*, 416-433 (1974).
WELSH, B. Ecology *56*, 513-530 (1975).

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